

# Isolation, Detection and Characterisation of Circulating Tumour Cells using Immunofluorescence

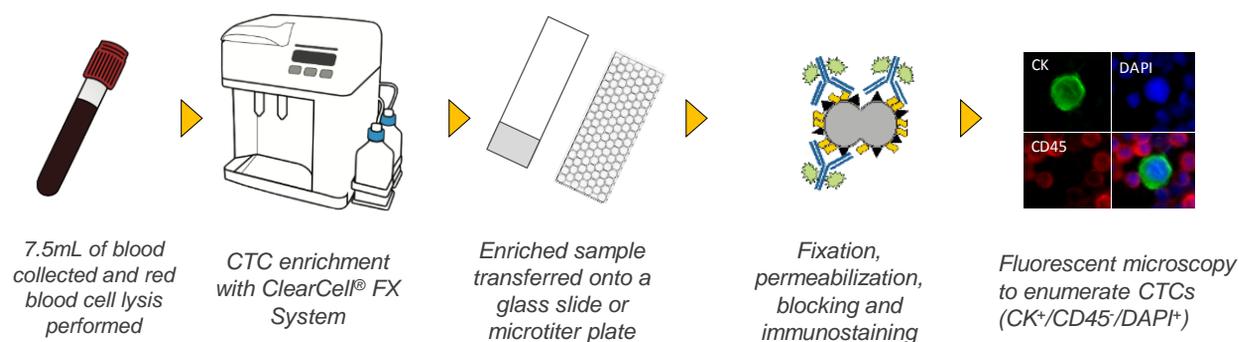
## Application Notes

### Overview and Key Results of ClearCell® FX

This application note describes the label-free enrichment of CTCs or microemboli from blood sample using the ClearCell® FX platform followed by immunofluorescence assay.

- ClearCell® FX enables label-free enrichment of CTCs and tumour microemboli from blood samples
- Sample output from ClearCell® FX is amenable for integration with immunofluorescence assay workflows
- Consistent recovery of CTCs ranging from 5 – 2000 cells per 7.5mL of blood
- CTCs and microemboli were enriched from lung, breast and prostate cancer patient blood samples

### Workflow for CTC enumeration



### Performance demonstration: advantages and consistency of ClearCell® FX

To demonstrate the advantages and consistency of label-free enrichment using ClearCell® FX, clinical samples were mimicked by spiking different concentrations (5 – 2000) of H1975 human lung adenocarcinoma cells (pre-labelled with CellTracker™) into 7.5mL of healthy blood (Fig. 1A). The results indicated a linear recovery of target cells across different spike in concentrations.

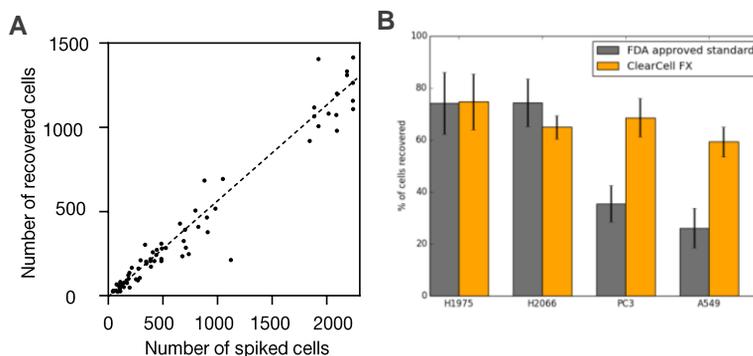


Figure 1. Validation of the system performance and precision of the ClearCell® FX.

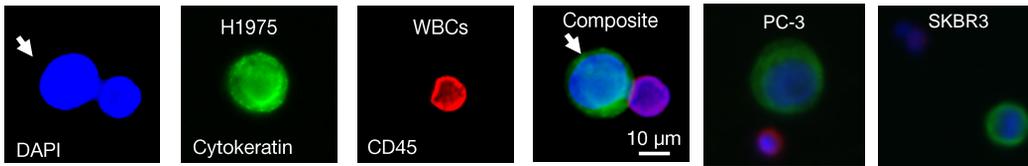
Both EpCAM-high and EpCAM-low cancer cell lines pre-labelled with CellTracker™ dyes spiked into healthy blood (H2066 and H1975 high, A549 and PC3 cells low) had recovery rates of over 59% (Fig. 1B) vs EpCAM based isolation platform. The use of a label-free enrichment platform such as the ClearCell® FX showed comparable recovery of EpCAM high expressing cells when compared to FDA approved standard and better recovery for EpCAM low expressing cells.

For more information please contact us at [support@clearbridgebiomedics.com](mailto:support@clearbridgebiomedics.com)

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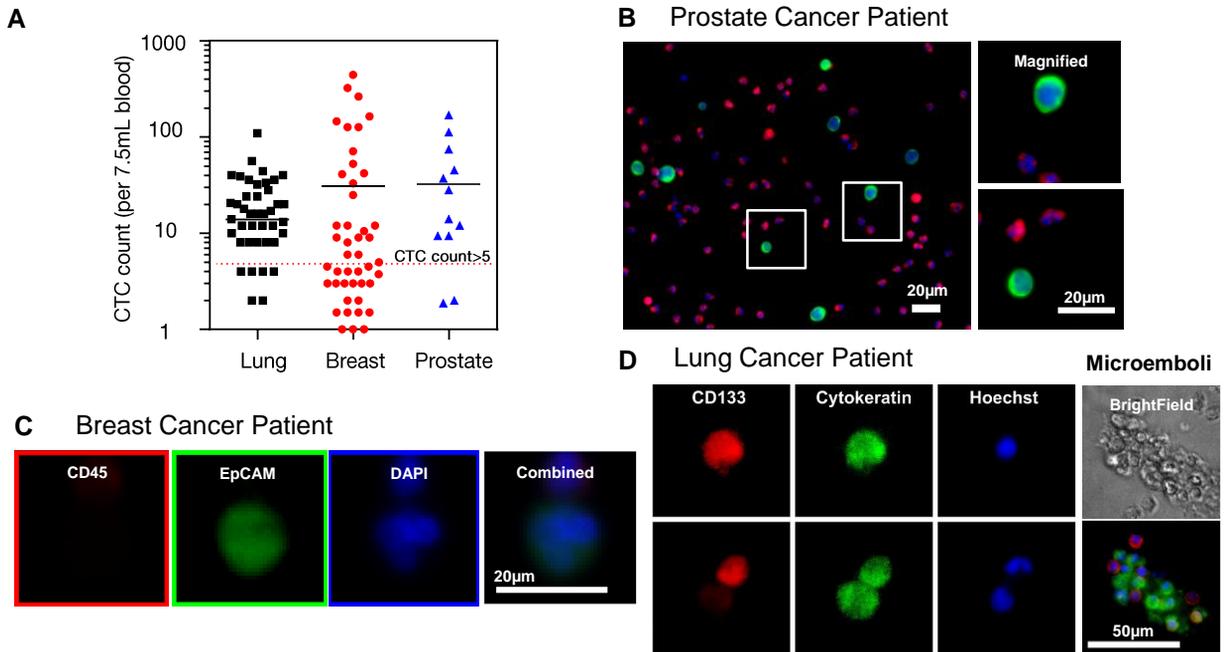
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### Immunofluorescence demonstration: Tumour Cell Lines H1975, SKBR3 and PC-3



Shown here are representative images of cancer cell lines H1975 (Lung), PC-3 (Prostate) and SKBR3 (Breast) identified using immunofluorescence staining. Cells were fluorescently labelled for pan-Cytokeratin, CD45 and DAPI.

### Intact CTCs and microemboli can be enriched from lung, breast and prostate cancer patients



(Hou, H. W. *et al.* cells using centrifugal forces, 2013 doi:10.1038/srep01259)

CTCs were enriched in 60 metastatic patients at various stages of lung, breast and prostate cancer. As the ClearCell<sup>®</sup> FX obviates the need of a single biomarker and samples a heterogeneous CTC population without bias, the number of CTC counts has a larger dynamic range from 0 to 382 as compared to EpCAM<sup>+</sup>-based approaches (Fig.2A). Immunofluorescent labelling of CK<sup>+</sup>, DAPI<sup>+</sup> and CD133<sup>+</sup> in metastatic lung, prostate and breast cancer patients indicated the presence of both single CTCs and microemboli after patient blood enrichment using the ClearCell<sup>®</sup> FX system (Fig. 2B-D). The characterisation of both single and microclusters, independent of EpCAM expression, were enabled through the unbiased recovery using the ClearCell<sup>®</sup> FX.

Find out more at  
[www.clearbridgebiomedics.com](http://www.clearbridgebiomedics.com)

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For more information please contact us at [support@clearbridgebiomedics.com](mailto:support@clearbridgebiomedics.com)